Isolation of *Clostridium perfringens* from three neonatal calves with haemorrhagic abomasitis

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SUMMARY

Braxy-like disease with sudden death and acute haemorhagic abomasitis was diagnosed in three Belgian Blue calves : one two-day-old and one one month-old calves, in good condition with no clinical signs noted a few hours prior to death, and another two day-old calf, which had shown problems of abomasal dilatation and regurgitation prior to death. Histologically, the abomasal wall were oedematous and emphysematous. A pure and abundant growth of *Clostridium perfringens* was obtained in anaerobic conditions from the abomasal wall of the three Belgian Blue calves. No bacterial growth was obtained in aerobic conditions. The calf with digestive disorders was also positive for BVD virus by immunofluorescence in the abomasal wall and in the spleen.

KEY-WORDS: calf - abomasitis - *Clostridium perfringens*.

RÉSUMÉ

Isolement de *Clostridium perfringens* chez trois veaux nouveau-nés souffrant d'abomasite hémorragique. Par C. MANTECA, T. JAUNIAUX, G. DAUBE, G. CZAPLICKI et J.G. MAINIL.

Un syndrome "Braxy-like", avec mort subite et abomasite aiguë hémorragique, a été observé chez trois veaux de la race Blanc-Bleu belge: deux veaux âgés de deux jours et un mois, en bonne santé et sans signes cliniques apparents quelques heures avant la mort, et un veau âgé de deux jours, avec une dilatation de la caillette et des problèmes de régurgitation peu de temps avant la mort. A l'examen histologique, la paroi des caillettes était oedématiée et emphysémateuse. Une culture pure et abondante de *Clostridium perfringens* a été obtenue en conditions anaérobies à partir de la paroi des caillettes de ces trois veaux. Les résultats des cultures bactériennes en conditions aérobies étaient négatifs. Le veau présentant des troubles digestifs était aussi positif par immunofluorescence pour le virus BVD à hauteur de la paroi de la caillette et de la rate.

MOT-CLÉS : veau - abomasite - *Clostridium perfringens*.

Introduction

Braxy, or bradsot, has been described in sheep for centuries [2]. Confusion with black disease was common until Gaiger [5, 6] demonstrated that the aetiological agents are different: braxy is caused by *Clostridium septicum* (*C. septicum*), whereas *C. novyi* toxin type B is responsible for black disease. Young sheep, less than one year in age, are at risk, especially in northern countries with cold climates (north of Great Britain, Scandinavia). Braxy is most often related to consumption of frozen food and is characterized by a very rapid evolution. Death usually occurs within two to twelve hours. The main lesion is an acute haemorrhagic abomasitis with wall oedema. *C. septicum* is located within the abomasum wall [4].

Braxy is suspected on the basis of the case history and main lesion. Confirmation is by Gram staining of a smear from the abomasum wall, and after bacterial growth and identification [11].

Only a few braxy-like cases have been described in young calves, and *C. septicum* does not appear to be the only aetiological agent [1, 4, 7, 10, 12]. This manuscript describes three cases of haemorrhagic abomasitis in young Belgian Blue calves, with identification of *C. perfringens* as putative aetiological agents.

Materials and methods

A) CALVES

Three calves were referred to the "Centre de Prévention et de Guidance vétérinaire, Province de Liège" for necropsy.

Calf #1 was a two-day-old male Belgian Blue calf referred two hours after death. No clinical signs were observed prior to death and his mother was healthy with no mastitis. The body was in good condition.

Calf #2 was a one-month-old male Belgian Blue calf originating from the same farm as calf #1 and was referred five months later. No clinical signs were observed two hours prior to death. The body was also in good condition.

Calf #3 was a two-day-old male Belgian Blue calf. Abomasal dilatation was observed after calving although the appetite was still good. An acute abdominal syndrome was observed 36 hours after birth with regurgitation and the calf died a few hours later.

B) POST-MORTEM EXAMINATION

Necropsy was performed following routine procedure. Organs with lesion were submitted to bacteriological, virological and, for some, pathological analysis.

Bacteria were grown in aerobic and anaerobic (anaerobic jars with generator; BioMérieux, Marcy l'Etoile, France) conditions at 37° C overnight, and for 48 hours if no growth was observed after 24 hours. Culture attempts for salmonella were performed in an enrichment broth (Merck, Darmstadt, Germany) for 48 hours and subsequently on Hektoen enteric agar plates (Sanofi Diagnostic Pasteur, Marnes, France). Suspect colonies were tested with commercial O:9 and O:4,5 immunsera (Sanofi Diagnostic Pasteur, Marnes, France) prior to identification. Bacteria were identified using appropriate API sugar sets according to the instructions of the manufacturer (BioMérieux, Marcy l'Etoile, France). For anaerobes API 32A were used.

Immunofluorescence assays were used to detect the presence of BVD virus. Positive results were confirmed by an ELISA (Synbiotic Corp., Lyon, France)

Tissue sections were performed following standard procedure and stained by Hematoxylin-Eosin and Gram methods. Gram staining was performed according to standard protocol.

Results

A) NECROPSY

All three calves had very similar to identical lesions. The mucosae with cyanotic. The most dramatic macroscopic lesion was an acute haemorrhagic non-ulcerative diffuse abomasitis with petechiae on the serous coat. Abomasal wall was oedematous and several times thicker than normal with a diffuse congestion. Emphysema was clearly visible internally in calf #1, but less so in calves #2 and #3. Some petechiae were also observed on the spleen and 500 mls to 1 liter of a serohaemorrhagic exsudate were present in the peritoneal cavity of the three calves.

B) BACTERIOLOGY

Short Gram positive rods were observed in Gram-stained smears from the abomasal wall of the calves. No salmonella was isolated and no bacterial growth was obtained in aerobic conditions. On the other hand, a pure and abundant culture of *C. perfringens* was obtained after growth in anaerobic conditions. *C. perfringens* was isolated in low numbers (<10 Colony Forming Units/ml) from the intestinal content. No bacterial growth was obtained from spleen, kidney or liver.

C) VIROLOGY

Immunofluorescence tests for BVD virus on spleen and abomasal wall were negative for calves #1 and #2, but were positive for calf #3. Results on calf #3 were confirmed by an ELISA assay on leukocytes and spleen.

D) PATHOLOGY

Tissue sections were performed on the abomasum of calves #2 and #3, and on the liver and kidneys of calf #2. Large zones of necrosis and short bacterial rods were observed in the abomasal mucosa of both calves; edema, emphysema and haemorrhages in the submucosa. Fibrin was also present in the abomasal submucosa of calf #3. No lesion was observed in the abomasal muscular coat of calf #2, but haemorrhages were present in the abomasal muscular coat of calf #3. No lesion or bacteria were observed in the liver and in the kidneys of calf #2.

Discussion

Description of braxy-like cases in calves caused by *C. septicum* is rare in the literature and those caused by other clostridia are even rarer [4, 11, 12]. The age of the animals varies from one week to three months. *C. septicum* is isolated most often alone or sometimes in combination with *C. chauvoei*, *C. perfringens*, or *C. sordellii*, but *C. perfringens* has also been associated on its own with ulcerative and haemorrhagic abomasitis in young calves [7, 10] and in adult cattle [1]. However, the evolution is slower and death was preceded by diarrhoea and tympanism.

Two of the three calves of this study were found dead with no other signs, a situation typical of braxy in sheep, and all had the standard lesion at necropsy, i.e. haemorrhagic abomasitis. Calf #3 suffered from abdominal disorders for two days prior to death, but this calf was also positive for BVD virus infection. Circumstances of occurrence (f.i. frozen food, overeating...) were not recorded.

If these three cases clinically resembled typical braxy in sheep, a pure and abundant growth of *C. perfringens* was

obtained from the abomasal wall, whereas *C. septicum* was not isolated. It may be argued that jarrs with generator systems do not create the best anaerobic conditions to allow the growth of *C. septicum*, which is not aerotolerant, in contrast to *C. perfringens.* However, no bacterial structure typical of *C. septicum* was observed in Gram-stained smears and tissue sections of the abomasal walls of the same calves. We can reasonably postulate that the aetiological agent is *C. perfringens* in the three calves. These *C. perfringens* isolates were not typed, but most probably belonged to the non-enterotoxigenic toxin-type A, as all *C. perfringens* isolates from Belgian Blue cattle tested so far belong to this toxin type [3, 9]. Alternatively, the BVD virus in calf #3 may be responsible for the clinical signs, lesions and death of the calf, although oedema and emphysema of the abomasal wall is not classically described in BVD disease.

Therapy of braxy is impossible due to its rapid progression, but prophylaxis based on vaccination as used in sheep is an alternative. Vaccination at 7 to 8 months of pregnancy was proposed in the farms from which the calves originated. No other cases were observed since, but the sporadicity of braxy disease in herds makes any conclusion unrealistic.

Braxy-like cases in calves may actually be under-diagnosed because sudden death in calves is often clinically diagnosed as "enterotoxaemia" [8], especially in beef calves, and no necropsy nor bacterial culture is performed. If more cases are examined, not only must *C. septicum* be regarded as an aetiological agent, but *C. perfringens* on its own as well.

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